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THE MICROGAMETOPHYTE OF THE PODOCARPINEÆ[1]

E. C. JEFFREY AND M. A. CHRYSLER

ALTHOUGH at the present time the views in regard to the relationships of the Coniferales depend very largely on the study of their gametophytic or sexual generation, our knowledge in regard to the gametophyte of the coniferous families is often very meager. The two families concerning which information is actually most needed are the Podocarpineae and the Araucarineae, exotics confined chiefly to the southern hemisphere. There is a prospect that our ignorance in regard to the Araucarineae will soon be less dense than it is at present, a consummation devoutly to be desired on account of the prevailing views, which make them the most ancient of the Coniferales. It is proposed in the present article to describe certain features of the male sexual generation of the Podocarpineae observed in material which we owe to the kindness of Dr. Cockayne of Christchurch, New Zealand, and Dr. Treub, Director of the Royal Gardens at Buitenzorg, Java. To both of these we tender our very warm thanks for the unfailing good nature which has made it possible for us to study some of the Australasian genera of the Podocarpineae. The material at our disposal was fixed in formaline or alcohol and consequently leaves something to be desired in the preservation of cytological details. As we shall however confine ourselves to the gross features of nuclear structure which do not suffer seriously by the methods of preservation described, this will not be a serious disadvantage.

The first species to be considered is *Podocarpus polystachya*, material of which we owe to Dr. Treub, Director of the Botanic Gardens at Buitenzorg. The male cones in our possession are in various stages of anthesis; but some of them show quite young anthers or microsporophylls in the upper region of the axis. This feature has made it possible for us to follow step by step the development of the male gametophyte up to the time of the shedding of the pollen or microspores, in spite of the fact that the material represents a single collection. *A* figure 1, represents the first mitosis in the microspore, which it will be observed is well advanced toward completion. The state of preservation of this material is remarkable in view of the fact that it was fixed in strong alcohol. In *b* figure 1, is to be seen the first prothallial cell fully formed and lying over against the upper or *posterior* side of the microspore. Beneath it, is the residual nucleus surrounded by vacuolated protoplasm. In *c* figure 1, is to be seen the mitosis which precedes the formation of the second prothallial cell. In *d* figure 1, the second prothallial cell is complete and lies against the first. At about this time the strongly thickened posterior wall of the microspore, which seems to be a peculiar feature of podocarpineous pollen, becomes markedly sculptured as is shown in *d*. In *a* figure 2, is shown a still later phase where the so-called generative cell has become added to the prothallial cells, which lie on the posterior wall of the microspore; it arises from another division of the residual nucleus. The contents of the pollen grain at this stage resemble in detail the conditions to be found in the abietineous microspore before the prothallial cells have begun to degenerate.

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[1] Contributions from the Phanerogamic Laboratories of Harvard University. No. 8.

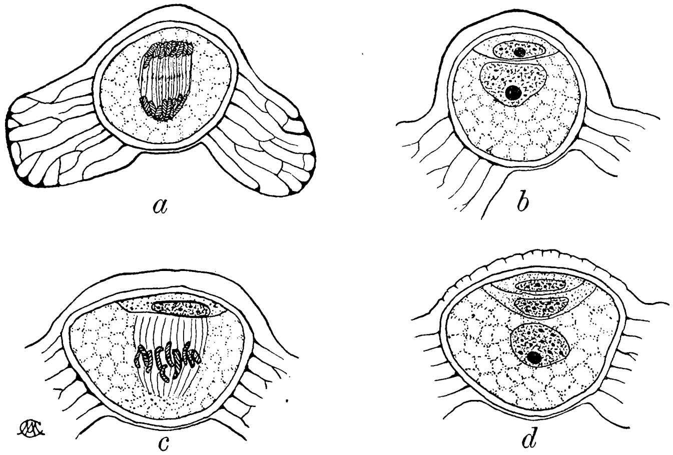

Fig. 1.— *Podocarpus polystachya.* *a*, First division of microspore. *b*, First prothallial cell cut off. *c*, Cutting off of second prothallial cell. *d*, Two prothallial cells cut off. × 925. The lateral air chambers, completely shown in *a*, are one of the numerous features of resemblance between the pollen of the Podocarpineæ and that of the Abietineæ.

In Podocarpus however there is no atrophy of the prothallial rudiments at this stage, but they undergo further changes of a surprising character, comparable only to those recently described by Thomson[1] in the genus Araucaria. In *b* figure 2, a later stage of development is shown, in which each of the prothallial cells has undergone transverse or anticlinal division. Division generally takes place first in the outer prothallial cell lying next the wall of the microspore and subsequently in the second prothallial cell. Contrary to the statements of Coker[2] in regard to *P. coriacea*, where a similar but less well marked condition has been described as a probable abnormality due to artificial conditions, anticlinal divisions of the prothallial cells are not initiated by direct division of the nucleus but by true mitosis. In the cells derived from

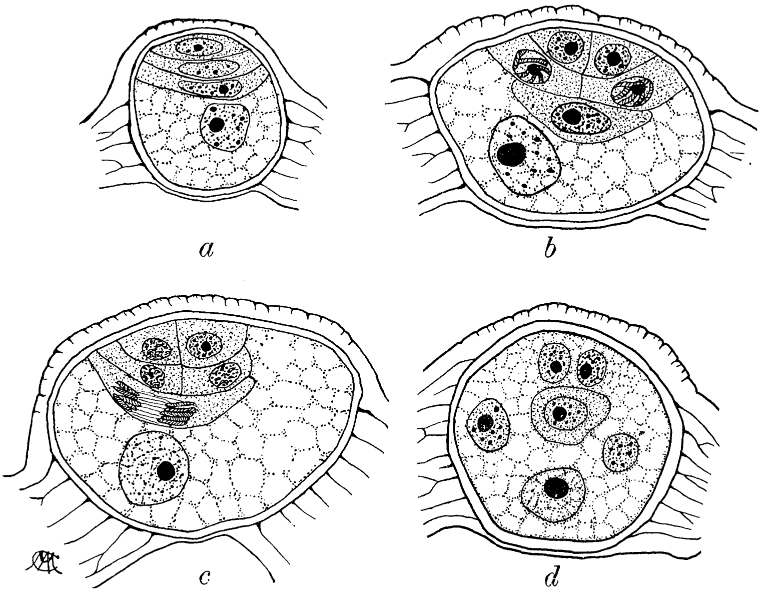

Fig. 2.— *Podocarpus polystachya.* *a*, Generative cell cut off. *b*, Anticlinal divisions of the two original prothallial cells. *c*, Mitosis in the first division of the generative cell. *d*, Nuclei of prothallial cells floating free in the cytoplasm of the pollen grain. × 925.

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[1] Thomson, R. B. The Araucarieæ — a 'Proto-Siphonogamic' method of Fertilization. Science N. S. **25**: 271, 272. 1907.

[2] Coker, W. C. Notes on the gametophytes and embryo of Podocarpus. Botan. Gazette **33**: 89–107. pls. 5–7. 1902.

the second prothallial cell in *b* figure 2, the nuclei are still in the spireme condition. Occasionally anticlinal divisions occur in the generative cell as in the prothallial rudiments. One such case is represented in *c* figure 2, which is an obvious and clear mitosis. Usually however in *P. polystachya* such divisions of the generative cell do not occur, although they are exceedingly common in some of the other species which we have had the opportunity of studying. At about this time the prothallial cells lose their walls; and their nuclei, floating freely in the cavity of the microspore, are no longer enclosed by cytoplasmic bodies. The nuclei, however, persist indefinitely and pass out as a swarm into the pollen tube. Among the unusually numerous free nuclei present in the microspore at this stage, the residual or tube nucleus can be distinguished readily by its large size, as is shown in figure 2 *d*; the generative cell, or the central cell derived from it in case it has undergone

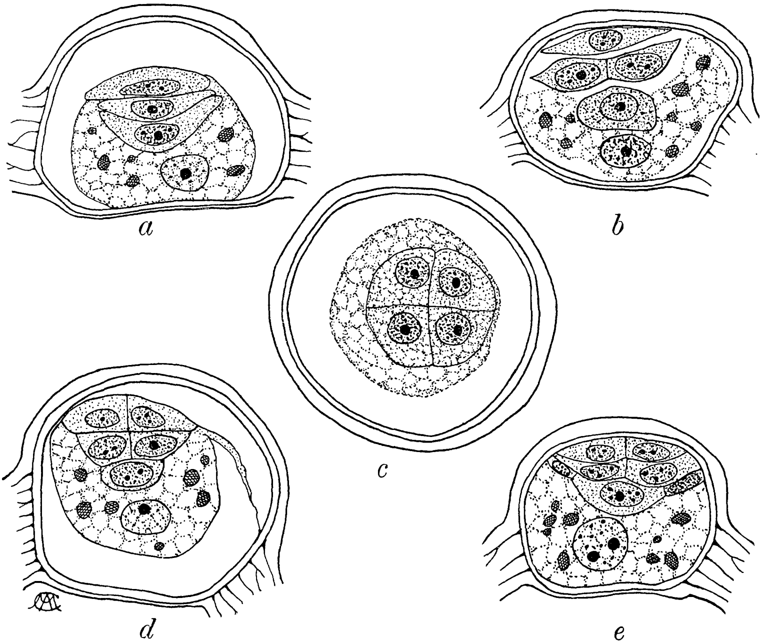

FIG. 3.— *Podocarpus ferruginea*. *a*, Two prothallial cells and generative cells formed. *b*, Anticlinal divisions of second prothallial cell. *c*, Tangential view of prothallial end of the gametophyte. *d*, Anticlinal divisions of both prothallial cells. *e*, Divisions in prothallial and generative cells. × 925.

anticlinal divisions previous to being set free from the prothallial complex, always retains its protoplasmic body as is generally the case in other Gymnosperms, and thus cannot be confused with any of the other contents of the microspore in the condition which immediately precedes anthesis.

In *Podocarpus ferruginea* from New Zealand, material of which we owe to the kindness of Dr. Cockayne, the earlier stages are not so well represented as in the species described above, but so far as they have been followed they present no essential deviation from the course of events in *P. polystachya*. *A* figure 3, represents the abietineous stage of development in this species. The preservation is even less good than that of the Podocarpus already described, and the protoplasm has shrunken from the microspore membrane. In *b* figure 3, is shown a fully developed grain, in which only one of the prothallial cells has undergone division. The generative cell in this case is also free from divisions, although it has rounded off and is almost ready to be set free from the cavity of the microspore. *C* figure 3, presents a tangential view of the first prothallial rudiment, which in this case has undergone two anticlinal divisions, so that four cells have resulted. *D* figure 3, presents a longitudinal section through the air chambers and shows anticlinal divisions in both of the prothallial cells. *E* figure 3, shows a similar condition in the prothallial rudiments; but in this case there are two lateral derivatives of the generative cell. The latter are very small in size compared with the central cell of the generative complex and with the derivatives of the prothallial rudiments.

As is represented in figures 3 and 4, starch is commonly found in the pollen grains, especially in the younger stages, though its presence is by no means constant. A similar feature has been noticed by Coker in the article cited above.

A figure 4, shows the structure of a ripe pollen grain in *P. dacrydioides*, from material sent us by Dr. Cockayne. The conditions are identical with those shown in *e* figure 3, representing *P. ferruginea*.

B figure 4, shows the gametophytic development in a probably mature microspore of *Dacrydium Bidwillii*, another representative of the Podocarpineæ. The material in this case proved to be very badly preserved. Dacrydium is distinguished from Podocarpus by the transverse striations of the thickened posterior wall of its microspore. In the species of Dacrydium which we have examined more than two prothallial cells are present, but the derivatives of the prothallial rudiments do not seem to be as numerous as they are in Podocarpus, where there may apparently be as many as eight present (*P. ferruginea*).

Through the kindness of Dr. Cockayne we have had the opportunity of comparing the microgametophytic development of Podocarpus and Dacrydium with that presented in Agathis, probably the more ancient of the two living genera of the Araucarineæ. In our material of Agathis the protoplasm is unfortunately very much shrunken, possibly on account of the small amount of alcohol in which it was preserved, but this fortunately does not interfere with the understanding of the general conditions present in the microgametophyte. In *b* figure 5, is shown an apparently mature microspore. We cannot however speak with certainty on this point, since none of the microsporophylls in our possession have shed their pollen. It is to be noted that the conditions present in this figure closely resemble those depicted in *b* figure 2, and *d* figure 3. In other words there are subsequent anticlinal divisions present in the two prothallial rudiments which are originally laid down as they are in the Abietineæ. *A* figure 5, resembles *b* closely and differs in the fact that only one of the prothallial rudiments has become divided. In *c* figure 5, is shown a tangential view of one of the prothallial cells. There have obviously been two anticlinal divisions in this case.

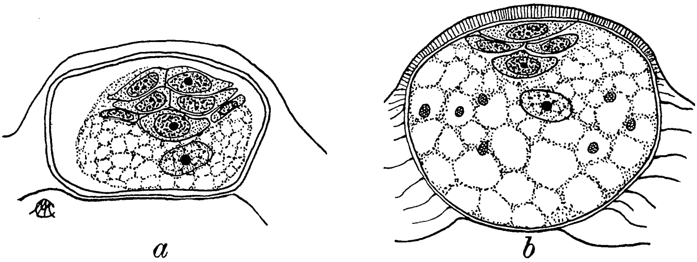

Fig. 4.— *a Podocarpus dacrydioides.* Divisions of prothallial and generative cells. *b, Dacrydium Bidwillii.* The second prothallial cell has divided. × 925.

It is apparent from the foregoing paragraphs that in two genera of the Podocarpineæ there are unusually numerous prothallial cells present in the microspore, which are derived by the subsequent anticlinal divisions of the two primitive prothallial cells. That these features are perfectly normal ones in the Podocarpineæ is made clear by the fact that all our material is from plants grown in their native habitat and presumably under natural conditions.

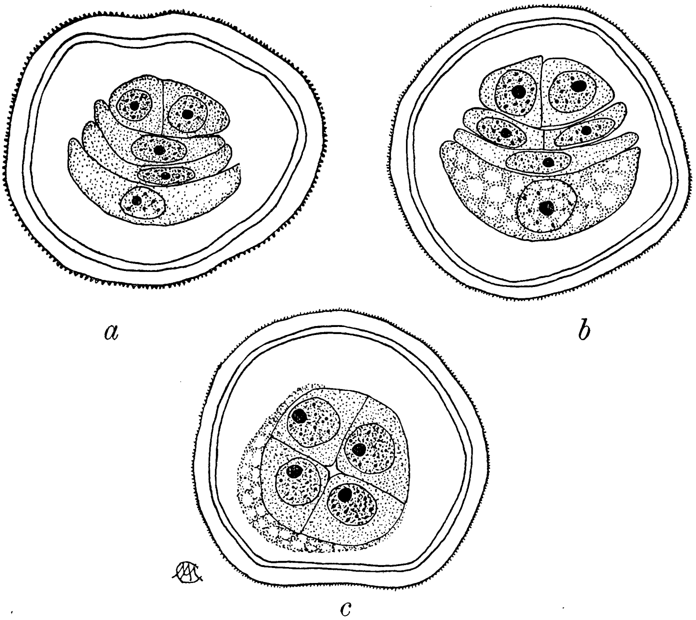

FIG. 5.— *Agathis australis.* *a*, The divided first prothallial cell, undivided second prothallial cell, generative cell, and tube nucleus. *b*, The same, but the second prothallial cell has also divided. *c*, Tangential view of the prothallial end of the gametophyte. × 925.

These features are further paralleled by the conditions presented by the microspore of the araucarian genus Agathis. The question here arises if we are to regard the rich prothallial endowment of the Podocarpineæ as the retention of a feature possessed by the ancestral Coniferales or as a recent cenogenetic adaptation, which has arisen at a later stage of evolution. This question can only be answered by a consideration of the microgametophytic conditions found in the Gymnosperms in general, particularly the more ancient of those still living. In the primitive zoidogamous

Cycads and Ginkgo there are one or two prothallial cells present. The generative cell undergoes only a tangential or periclinal division in connection with the formation of the stalk cell and antheridial cell. The antheridial cell in both the Cycadales and Ginkgoales gives rise to two spermatocytes, the mother cells of antherozoids. In the Abietineæ, which we know from the evidence of the fossil remains extend very far back geologically in forms allied to Pinus, there are two evanescent prothallial cells present in the mature microgametophyte, and a generative cell which as in the zoidogamous Gymnosperms gives rise to stalk and antheridial cells by periclinal division. The antheridial cell in turn gives rise to two cells which are to be regarded as the homologues of the two spermatocytes of the Cycadales and Ginkgoales. In the Araucarineæ, so far as our knowledge goes, there are formed at first two prothallial cells, which may subsequently undergo more or less numerous anticlinal and possibly also periclinal divisions. The final history of the generative cell is obscure, but it is to be inferred from the brief summary of Thomson (*loc. cit.*) that the antheridial cell of the Araucarineæ does not divide into two as in the Abietineæ and the ancient zoidogamous Gymnosperms. In the Araucarineæ there is a further remarkable feature in that the pollen grain does not reach the micropyle of the ovule as in the other Coniferales and all other known Gymnosperms living or fossil; but is deposited on some part of the ovuliferous scale or megasporophyll (on the 'ligule' in Araucaria) thence sending a pollen tube down to the ovule, in a manner analogous to that obtaining in the Angiosperms. Thomson, adopting the prevailing hypothesis that the Araucarineæ are the most primitive Coniferales, designates this peculiar mode of fertilization as primitive or 'protosiphonogamic.'

This view presents some difficulties, for if the quasi-angiospermous method of fertilization found in the Araucarineæ is 'primitive' it is difficult to see why such a method is entirely absent in the older gymnospermous series, the Pteridospermæ, Cordaitales and Ginkgoales, or being ancestral for the Coniferales is entirely lost in the coniferous families other than the Araucarineæ, which have moreover a method of pollination resembling closely that of the older Gymnosperms in that the microspores are received through the micropyle. The reported presence of only a single sperm-cell in the Araucarineæ supplies another argument against their being more primitive than the other Coniferales. Their superior antiquity further does not rest on any sound palæontological basis, for so competent an authority as Schenk (Zittel's Handbuch) remarks that if more abundant and more ancient geological occurrence were to be considered as a criterion of antiquity, the Araucarineæ must yield place to the Taxodineæ. It appears not unlikely, especially in view of observations made by one of us on Mesozoic Coniferales, shortly to be published, that the 'protosiphonogamic' method of fertilization which is the interesting discovery of Mr. Thomson, is correlated with the proliferation of the prothallial cells in the Araucarineæ, since the greater length of pollen tube, in the absence of any special conductive tissue such as is found in the Angiosperms, calls for a greater development of prothallial tissue. The failure of the pollen to reach the micropyle, on the other hand, may have been due to the unfavorable influence of drought upon the fluid secretion which in other Conifers floats the pollen to the micropyle.

Turning from the Araucarineæ to the Podocarpineæ, we find very similar conditions in regard to the prothallial proliferations. The plan of prothallial development here as in the Abietineæ and Araucarineæ involves two prothallial cells, but as in the Araucarineæ these have apparently undergone cenogenetic proliferation. That this is the true view of the matter is rendered more probable by the fact that even the generative cell may be affected by the process of proliferation, as in *Podocarpus polystachya, P. ferruginea* and *P. dacrydioides,* described above. There is certainly no reason from our knowledge of the older and zoidogamous Gymnosperms to regard the anticlinal proliferation of the antheridial cell as a primitive feature, since so far as our present information goes such a phenomenon is quite absent here. Further whatever prejudice there may exist in favor of the Araucarineæ being a primitive family of Conifers, there can be none in favor of a like view in the case of the Podocarpineæ. The development of the microgametophyte in the case of the Podocarpineæ as here described only serves to strengthen the opinion already expressed by Coker (*op. cit.*) and Thomson[1] that they are not very remotely connected with the Abietineæ. Their peculiar prothallial developments represent an apparently cenogenetic superaddition to the primitive type of coniferous microgametophyte found in the Abietineæ. If this view be taken of the position of the Podocarpineæ, it may well be extended to the Araucarineæ which present a similar microgametophytic development, although it would take us too far afield and would involve the discussion of yet unpublished data in regard to living and fossil Coniferales, to defend that proposition in the present connection.

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[1] Thomson, R. B. The megaspore-membrane of the Gymnosperms. Univ. of Toronto Studies, Biological Series No. 4. 1905.

SUMMARY

1. The Podocarpineæ as represented by the genera Podocarpus and Dacrydium are characterized by a proliferation of the two original prothallial cells through more or less numerous anticlinal divisions.
2. The anticlinal proliferation of the prothallial cells in some cases is accompanied by a similar proliferation of the generative cell, an abnormality which appears to have been described in no other Gymnosperms.
3. Similar proliferation of the two original prothallial cells has been observed in the araucarian genus Agathis.
4. The proliferation of the two prothallial cells in the Podocarpineæ and Araucarineæ and the proliferation of the generative cell in certain species of Podocarpus, cannot be regarded as a primitive feature.
5. The ground plan of microgametophytic development found in the Podocarpineæ and Araucarineæ points to their derivation from an ancestral stock allied to the Abietineæ.
6. Since the Podocarpineæ and Araucarineæ present many features of similarity in general habit, in geographical distribution, in the organization of their megasporophylls, and the development of their microgametophytes, it seems not improbable that they are somewhat more nearly allied than has been supposed.

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